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AN EXPERIMENTAL STUDY OF THE PHYSICAL CHEMISTRY OF ANAESTHESIA IN RELATIONSHIP TO ITS CAUSATION

BY

BENJAMIN MOORE AND HERBERT E. ROAF

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AN EXPERIMENTAL STUDY OF THE PHYSICAL CHEMISTRY OF ANAESTHESIA IN RELATIONSHIP TO ITS CAUSATION

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Following closely upon this observation of DURHAM, BEDFORD-BROWN[1] demonstrated in the same year by observations on a patient having the brain surface exposed, as the result of accident, that the induction of anaesthesia caused a primary hyperaemia which soon passed off and was succeeded by anaemia.

Contradictory observations followed on this by various observers, some of whom found hyperaemia and some anaemia during anaesthesia, but a critical research by CLAUDE BERNARD[2] demonstrated the accuracy of BEDFORD-BROWN's observations, and there is now little doubt that the observations of hyperaemia were due to the presence of inflammation, the result of unskilful trephining, and not to the action of the anaesthetic. But while it may be taken as established, from these observations, that both sleep and anaesthesia are accompanied by anaemia of the brain, it is obvious for various reasons that this can only be a secondary factor or accompaniment, and not the primary cause of either condition.

In the first place, anaesthesia can be produced in organisms in which there is no blood supply and even no nerve cells, for anaesthesia is a condition which can be impressed upon any living cell no matter how lowly its organization. Hence the primary action must be directly upon some constituent of the cell and not solely upon something outside, although in the case of the higher cells, such as the cortical cell of the mammal, interference with its nutrition by alteration in blood supply may act as such a cause and produce anaesthesia. There is no doubt whatever that suddenly produced anaemia of the brain, such as occurs, for example, by double compression of the carotids, by vagus action upon the heart, or by the sudden hydrostatic fall in cerebral blood pressure caused in debilitated individuals by assuming the standing position, gives rise at once to an anaesthetic effect and to sudden loss of consciousness. But here again the cause of the anaesthesia is something of a chemical nature suddenly occurring in the nerve cells, the result of the stoppage or alteration in the cerebral circulation, and not a merely mechanical effect produced by the fall of pressure.

Secondly, although the lessened blood flow in the brain may assist in maintaining sleep or anaesthesia, by lessening the metabolic changes in the nerve cells, it is quantitatively too slight, acting alone, to produce the whole effect in this manner.

Thirdly, even admitting that a part of the effect in both sleep and anaesthesia may be due to altered blood supply, there remains to be sought out the cause of the altered blood supply. This, whether it be a direct effect upon the musculature of the blood vessels or be due to an indirect effect through the vasomotor system, must in the end arise from some chemical effect of the anaesthetic upon muscle cell or nerve cell.

Hence, while there is probably similarity in mode of causation of sleep (including coma) and anaesthesia, such effects as variations in blood supply can only be set down as secondary factors, due also to the same primary cause as directly acting upon the nerve centres, produces the unconsciousness of sleep, of coma, or of anaesthesia.

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[1] *American Journal of Medical Science*, Oct. (1860).

[2] Claude Bernard, *Leçons sur les Anaesthèsiques* (1875), p. 117.

It is clear then that we must turn to a consideration of the effects of the anaesthetic upon such constituents as are present in the cell, and as a preface to the consideration of the various theories which have been put forward, it may be well to point out that although anaesthesia in mammals means the abolition of consciousness, and the accompanying incapacity for the production of sensation following peripheral stimulation of nerve endings, yet this effect only differs in degree from other effects which appear as the concentration of the anaesthetizing agent is gradually increased. The cortical nerve cells pass first under the influence of the anaesthetic, while other portions of the nervous mechanism are still active and amenable to stimulation. For example, the reflex nervous mechanisms throughout the medulla and cord are still active, such as those which regulate the respiratory and cardiac rhythms, peristalsis, the condition of the vasomotor system, and the sphincters throughout the body, although the degree of activity is, in most instances, depressed.

If the concentration of the anaesthetic be increased, however, these remaining portions of the nervous system also pass under the action of the anaesthetic, and death of the mammal ensues, usually from stoppage of the action of the nerve cells responsible for the continuance of the respiratory rhythm.

Nor is it the cells of the nervous system alone which are affected by anaesthetics, for at increasing concentrations isolated mammalian tissues are affected, and their activities inhibited and finally stopped, as has been demonstrated experimentally for nerve fibres, for cardiac and skeletal muscle, and for ciliated cells.

The same effects are demonstrable not only for mammalian tissues, but also in all living cells of whatever source, whether of vertebrate or invertebrate, of animal or plant, of the lowliest unicellular organism or of the highest mammal.

This result is of importance, as clearly showing that the effect of the anaesthetic throughout the whole range of living cells is produced in some common fashion upon a constituent which is uniformly present in every living cell, and is not something peculiar to the nerve cell, nor explicable by some morphological arrangement present in the nerve cell and not present in other cells.

The result teaches, for example, that no faith can be placed in a mechanical theory of anaesthesia, such as the withdrawal or protusion of the dendrites of the nerve cell, for this would yield no explanation of the similar action of anaesthetics upon other types of cell.

The universality of the action of anaesthetics upon all living structures further narrows the field of search for the substance upon which the anaesthetic acts, since any occasional cell constituent may at once be removed from consideration, and only those left which are invariably present.

When this view has been taken, the material which at once occurs to the mind is that which is most intimately connected with the activity of the cell, and is always present, namely, the cell-protoplasm, or, since we are considering the matter from the chemical point of view, the organized proteids which go to build up the cell.

In addition to the proteids, there is another substance, or rather group of substances, widely distributed throughout the world of living cells, namely, the lecithins and allied bodies which have recently received the name of 'cell lipöids.'

If by the term 'cell lipöids' is to be understood anything soluble in ether, so including bodies so widely dissimilar in nature as neutral fats, cholestearin, and lecithin, it must be admitted that all cells contain 'cell lipöids,' although in many cases, even with this wide and somewhat meaningless definition, the amount present, compared to the proteid and other organic constituents, is infinitesimally small in many types of cell. But if the term is taken to mean lecithin and its compounds only then there exists no experimental próof that 'lipöids or lecithins' are present in appreciable quantity in every cell, and exception must be taken to the important position which has recently been assigned to these so-called lipöids in governing such a fundamentally important process as selective absorption, or as agents in producing anaesthesia by virtue of their high solvent power for anaesthetics.[1]

The earliest experiments which we have been able to trace regarding the action of chloroform and other anaesthetics upon tissues, other than those of the nervous system, have reference to an action of chloroform upon the skeletal musculature which was noticed soon after the introduction of anaesthetics, and attracted the attention of a number of observers. The experiments were made upon the effects produced by injection of chloroform into the arteries supplying the limbs, and the results obtained were a strong contraction of all the muscles, which, with larger quantities, remained permanent, the muscles becoming opaque and stiff, as in the case of heat rigor, or *rigor mortis*.

Although the experiments were carried out with quantities of chloroform greater than are required for anaesthetic purposes, they demonstrate an action upon the muscle substance which is interesting from the point of view of the experiments which we have to describe later in this paper.

The first observer who appears to have worked upon the subject was Coze,[2] who found that after injection of a few drops of pure chloroform into the crural artery of a rabbit, there followed immediately a muscular contraction so pronounced that the muscles appeared to have acquired the hardness of wood. The rigor only slowly disappeared if the animal survived the experiment, but in the end the voluntary movements of the limb became partially restored. Similar rigor was induced by injection of ether, but larger quantities were required to produce the effect.

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[1] Overton, *Vierteljahrssch. d. Naturf. Gesellsch. in Zurich* (1899), Bd. xliv, S. 88-135; *Studien über die Narkose, Jena*, Gustav Fischer (1901).

[2] *Comptes rendus de l'Acad.*, xxviii, 1849, p. 534.

Brown Sequard,[1] in 1853, confirmed Coze's observations, and added that circulation of fresh arterial blood through a limb which had been rigored by chloroform caused a return of excitability even after a period of two to ten days.

Küssmaul[2] independently repeated and confirmed Coze's work, and was first to point out that the condition was one allied to rigor and not to tetanus; he also showed that, although chloroform caused precipitation in fresh extracts of muscle, the main action of the anaesthetic was upon the substance of the muscle cell. Küssmaul was unable to repeat Brown-Sequard's observation as to recovery of irritability after prolonged action of chloroform.

Ranke[3] found that frog's muscle after the long-continued action of chloroform vapour upon the animals became rigid, and further showed that this was, in all probability, due to a coagulation of the myosin, as he observed a turbidity in clear solutions of the muscle proteid after subjecting to chloroform vapour for a period of three quarters of an hour. Ranke also obtained a similar turbidity, but in lessened degree by the action of the vapours of alcohol, ether, and amyl alcohol upon 'myosin' solutions.

Buchheim and Eisenmeyer[4] later subjected frogs to saturated chloroform vapour for an hour, and failed to to get Ranke's muscular rigidity. They found that under such circumstances all movements and the respiration stopped, and the frogs appeared to be dead. The muscle curves taken from the muscles after this treatment showed a short latent period and a normal period of contraction, but the relaxation was veratria-like, and only approached completion after twenty-five to thirty revolutions of the cylinder.

Claude Bernard in his *Leçons sur les anaesthesiques*, published in 1875, recurred to these experiments on the rigidity and semi-coagulation of skeletal muscle when subjected to the action of chloroform vapour, and suggested that an analogous semi-coagulation probably occurs in nerve cells, and that anaesthesia may be brought about as a result of such a coagulation which may not be definite and fixed, that is to say, in which the cell substance can return structurally to its primitive state after elimination of the toxic agent.

A similar view was expressed by Binz,[5] who stated that sections of cerebral cortex placed in the one per cent. solution of hydrochlorate of morphia soon showed a cloudy appearance, and fine granules appeared in the nuclei; the protoplasm also became granular. The stage at which the cell-protoplasm was merely cloudy, and not discretely granular, could be recovered from by washing away the morphia, but, when once the granules appeared, they could not be made to disappear again. Similar results were obtained by exposing cortical nerve-cells to vapour of chloroform, or to solution of chloral hydrate.

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[1] Canstatt's *Jahresberichte*, 1853, s. 199. *Experimental Researches Applied to Physiology and Pathology*, New York, 1853.

[2] *Arch f. Path. Anat.*, Bd. xiii, 1857, S. 289.

[3] Buckhner's *Repertorium*, Bd. xvi, S. 374.

[4] Eckhardt's *Beiträge* (1869).

[5] *Vorlesungen uber Pharmaklogie*, S. 175.

Neither **Binz** nor **Bernard** showed that there was any precipitation or semi-coagulation at or near the concentrations which correspond to anaesthesia, nor were the optical methods used capable of demonstrating effects upon the protoplasm short of precipitation.

Dubois[1] advanced the theory that anaesthetics produce their effect by a partial dehydration of the protoplasm, by increasing the tension of dissociation between the water and the tissues, as he put it, so that the latter part with a portion of their water, and so tend to approach the dormant condition of dried seeds and other organisms in the dehydrated condition. **Dubois** quotes an experiment in which plant tissues on being subjected to the action of saturated chloroform vapour exuded water, and compares the effect to that produced by cold. There is, however, nothing convincing in the experiments, and the transudation of water is more probably due to injury of the epidermal cells than to any physical action of the anaesthetic directly upon the protoplasm.

Schmiedeberg,[2] in his *Grundriss der Pharmakologie*, states that chloroform retards the passage of the oxygen from the oxyhaemoglobin to easily oxidizable substances and forms a peculiar compound with the haemoglobin, but that these changes cannot be proved to take place in the living body.

Turning from the theories which drive anaesthesia from an action of the anaesthetic upon the cell protoplasm to those which, inspired by the large percentage of bodies soluble in ether which the nervous system contains, look for an explanation to some effect upon the cell lipöids, we find the view advanced by **Bibra** and **Harless**[3] soon after the discovery of the anaesthetizing action of chloroform and ether that these anaesthetics owe their activity to their solvent power for the fatty and allied constituents of the nerve cell.

These authors supposed that the anaesthesia was produced because the anaesthetic dissolved out a portion of the lipöid substances, and the experimental evidence on which they based this hypothesis was that after repeated anaesthetization by ether, analysis showed that the ethereal extractives of the brain decreased in amount, while the amount of ethereal extractives in the liver increased.

It is, however, most probable that this result was due to an experimental error, for if there were any actual removal of bodies soluble in ether from the nerve cell, it is difficult (1) to see how the removal could be effected by such small quantities of anaesthetic as are required for the induction of anaesthesia acting merely in dilute solution in the plasma; (2) to see how removal of a small fraction of the total lipöid could produce anaesthesia; and (3) to explain if the removal of this small fraction of lipöid does produce anaesthesia, how the anaesthesia can be so quickly recovered from when administration is interrupted; for the hypothesis presents no mechanism for the rapid restoration of the materials supposed to be removed during anaesthetization. The last argument also disposes effectually of any theory whatever which depends upon a removal of any permanent element of the cell as a cause of anaesthesia.

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[1] *Anaesthésie physiologique*, 1894, p. 15.

[2] Quoted from the authorized translation by Thomas Dixon, M.B., Young J. Pentland, Edinburgh, 1887.

[3] Quoted from Overten, *Studien über die Norkose*, S. 50.

A somewhat reversed theory to that just discussed which has lately gained much support was put forward independently and almost simultaneously by HANS MEYER[1] and E. OVERTON.[2]

Instead of ascribing the action of anaesthetics, such as chloroform and ether,[3] to the solvent action of the anaesthetic upon the lipöids of the cell, the basis of the theory of MEYER and OVERTON is that the cell lipöids dissolve or take up physically the anaesthetics, by which process the physical properties of the lipöid are altered, and in some manner anaesthesia is induced as a result of this alteration in the lipöids.

The authors leave the manner in which this supposed physical alteration in the properties of the lipöids, by the taking up of the anaesthetic, brings about anaesthesia an open question. OVERTON suggests that the lipöids are either unable to carry out, in this altered condition, their own functions within the cell, or that the new physical conditions of the lipöids acts as a disturbing influence upon the functions of other constituents of the cell.

In regard to the nerve cell, OVERTON further throws out the surmise that the alteration in the condition of the lipöids by the anaesthetic may increase the physiological resistance to the passage of the stimulus both within the cell, and between one cell and another, and also that the activity of the cell to respond when a stimulus reaches it may be lessened by the presence of the anaesthetic in the lipöid.

Although a large number of substances have been examined by MEYER and by OVERTON from the point of view of the relationship of their strength as anaesthetics to their relative solubility in water and in olive oil, and it has been demonstrated *roughly* that the relationship exists, that the less soluble any given substance is in water, and the more soluble it is in oil, the more powerful it is as an anaesthetic. Yet, it may be pointed out, that this by no means settles the matter, for there are other substances in the cell than the lipöids, and it must also be shown, before the argument gathers any weight, that a similar physical property does not belong to any of these, that there are no other substances in the cell which have similar affinities along parallel lines for taking up the anaesthetics, and which may be more intimately connected with the activities of the cell than the lipöid constituents.

It is clear that if the anaesthesia be due to any substance whatever in the cell, combining physically or chemically (in unstable combination) with the anaesthetic, that the concentration of the anaesthetic in that substance of the cell must vary inversely as the solubility of the anaesthetic in water, because the anaesthetic distributes itself always according to its relative solubilities in the two media between which it is distributed.

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[1] Sitzungsber., *d. Gesellsch, z. Beförderung d. ges. Naturwissen*, Marburg, 1899. *Arch. f. exper. Path. u. Pharm.* Bd. X C ii, S. 109; see also F. Baum. *Ibid.* S. 119.

[2] Overton, *Studien uber die Narkose*, G. Fischer, Jene, 1901.

[3] Anaesthetics of this class are designated as indifferent anaesthetics by Overton in contra-distinction to basic anaesthetics, which include such of the alkaloidal anaesthetics as possess basic properties. According to this observer the action of the latter class is different in origin, and probably due to interaction with the cell proteids.

This, we maintain, is all that is shown by the experiments of Meyer and Overton, who have not experimented with the lipöids, but with olive oil, to which no one attributes the production of anaesthesia, and we regard it as dangerous to transfer the results of experiments made with olive oil and water to lecithin or other lipöids, and water[1].

Again, determinations have not been made, except in a few cases, of the coefficient of distribution of the anaesthetic between even olive oil and water, but instead, only rough determinations of the maximum solubilities in these two media. Also, one meets in, by far, the greater number of Overton's experiments with the statement that the anaesthetic, under experiment, is soluble in all proportions in one or other of the two solvents, usually the olive oil. In fact, throughout whole tables of comparison of different anaesthetics belonging to the same organic group, it is observable that the whole of the substances tested are soluble in olive oil in all proportions, and hence, there is no means of knowing what influence relative solubilities in olive oil possess since such relative solubilities do not exist. Under such circumstances it is impossible to tell how the coefficient of distribution is varying, for, of course, the fact that an anaesthetic is soluble in all proportions in olive oil, and has only a low solubility in water, does not indicate that the coefficient of distribution between olive oil and water is infinity. For example, quoting the figures given by Overton[2] for two cases, ether is soluble in all proportions in olive oil, and dissolves to the extent of 6·6 per cent. in water, yet its coefficient of distribution between oil and water is only 4·5 ; chloroform is soluble in all proportions in olive oil, and in water to 0·72 per cent., yet its coefficient is only 30—33.

The observation that the anaesthetizing power of indifferent organic substances such as chloroform and ether vary inversely as their solubility in water had already been made by Richet, and is referred to by Overton[3] as *Richet's rule* ; but Richet thought the explanation a different one, and ascribed the action to the fact that such substances being but feebly soluble in water, did not normally enter into the cell or act upon it, and hence exerted a toxic action when they did enter. This explanation, however, does nothing towards elucidating what happens when these substances do enter the cell, nor is there any proof that diffusion into the cell is difficult for such substances, but rather the reverse is true, for the facts of the rapid onset and recovery from anaesthesia show that they enter and leave the cell quite freely and rapidly.

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[1] The explanation given for the use of olive oil, instead of the lipöids, or lecithin, is, that the latter are so difficult to isolate in sufficient quantity for the experimental determination of the coefficients of distribution ; but an ethereal extract of brain can easily be obtained in large quantities, and lecithin can now be isolated in large quantity, and with, comparatively, little expense from either brain or egg yolk (see paper succeeding this in the present volume, p. 201). A real difficulty in determining these coefficients in the case of lecithin or the lipöids, lies in the physical properties of these bodies, which are not fluids, but plastic solids, and hence it is not possible to bring a mixture of these and water into equilibrium with such solvents as chloroform, etc., and then to determine the fractions of the chloroform, etc., in the lecithin, or lipöids, and in the water respectively. The difficulty is still further increased by the swelling up and suspension of the lipöids, or lecithin, in the water.

[2] Overton, *Studien uber die Narkose*, SS. 96 and 100.

[3] *Ibid.*, S. 45

With regard to the statement that in such a large number of cases the anaesthetic is not only less soluble in water but also more soluble in olive oil, it may be pointed out, that in the vast majority of the substances experimented with the substances are soluble in all proportions in olive oil, and hence no comparison is possible. Secondly, it is a general rule in the case of all organic substances of the classes under consideration, that the less soluble they are in water the more soluble they are in organic substances, which, like themselves, are insoluble in water.

For example, if a given organic substance has a low solubility in water and a high solubility in, say, ether, in the great majority of cases it will also have a high solubility in chloroform, benzol, xylol, toluol, and the oils and fats, viz., in substances which have themselves a very sparing solubility in water.

Finally, it may be mentioned that the lipöid theory does not at all fit the experimental facts in the case of the basic anaesthetics[1], or narcotics, which belong to the class of bodies known as alkaloids, such, for example, as coniin, nicotin, and morphia. For this class of compounds, OVERTON[2] admits the existence of combinations, probably of the nature of salts between the basic anaesthetic and the tissue proteids, and it is strange that this should not have suggested determinations of the action of proteids upon the so-called 'indifferent' anaesthetics, which would have at once demonstrated that these form similar compounds, as is shown by the experiments detailed below.

It hence becomes an advantage of the theory which refers the causation of anaesthesia to the formation of unstable combinations between the anaesthetic and the cell protoplasm, that it furnishes a common explanation of the action of all types of anaesthetic whether basic or indifferent.

A basis is also provided for an explanation of the difference in rate of recovery from the anaesthesia in the case of the different anaesthetics, and for the selective action of certain anaesthetics. For it is obvious that such factors will have an influence as the stability and dissociation pressure of the combination between the anaesthetic and the protoplasm, as well as the rate of diffusion into and out of the cell, in determining the rate of intake and output of the anaesthetic, and hence of the onset and subsidence of the anaesthesia. Further, there must exist differences in the composition and state of aggregation of the proteid molecules going to build up the protoplasm in the case of different classes of cells, and hence facility or the reverse for combination with different chemical compounds or anaesthetics must exist, so that one alkaloid will accordingly pick out and combine with the organized proteid of one type of cell, leaving others free, while another anaesthetic or alkaloid will form combinations in a different type of cell and so produce a different physiological effect.

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[1] There are also certain exceptions in the case of the so-called indifferent anaesthetics.

[2] *Studien uber die Narkose*, S. 160, et seq.

Considered from this point of view, the basic anaesthetics which (as shown by the greater slowness with which their effect disappears) form more stable compounds will be more specific in their action, while the indifferent anaesthetics or, as they might perhaps better be called, *general* anaesthetics such as ether and chloroform, which form much more loose combinations, probably of a physical character with the proteid of the protoplasm, will be adapted for more general combination, and will affect all cells more equally, although even here, as has already been pointed out, there is selective action in lessened degree.

We may now pass from the history of the subject to a description of our own experiments, which have chiefly been made with chloroform, although certain experiments have been added with other anaesthetics, which show that the conclusions deduced from the experiments with chloroform hold also for other anaesthetics, and at the outset we desire to acknowledge that our attention was first attracted to the subject by witnessing the experiments of SHERRINGTON and SOWTON[1] upon the effects of chloroform on the excised mammalian heart, fed by a current of RINGER's or LOCKE's solution, and through which, later, a similar current, but containing in addition small amounts of chloroform, could be perfused.

These authors observed that a concentration of chloroform in the LOCKE solution, amounting to only 1 in 100,000, produced a marked and unfailing action in diminishing the extent of the cardiac contractions, and, further, that this effect appeared rapidly after the dilute chloroform solution reached the heart, lasted just as long as the chloroform in this excessively low but yet adequate concentration was passed through, and ceased almost immediately as soon as the normal LOCKE's solution was recurred to, the heart attaining again its normal force.

This effect could be repeated as often as was desired, and there was no cumulative action whatever, that is, no matter how prolonged the passage of the chloroform solution on passing back to the normal LOCKE's solution, the chloroform effect rapily disappeared, and again recovered when the chloroform was once more turned on.

It was this latter effect which suggested the experiments on the chemistry of anaesthesia recorded in this paper. It was quite obvious that the effect of the chloroform upon the cardiac muscle fibres depended solely upon the concentration (solution tension or osmotic pressure) of the chloroform in the cell for the time being, and not at all upon the total amount of chloroform which had been fed to the heart up to the moment of observation.

This experimental fact suggested the view that the effect upon the cell was due to some combination being formed between the protoplasm and the chloroform, and further, that this combination was not a stable fixed one, leading to permanent removal of the protoplasmic activity, but an unstable one, which existed only so long as the pressure of the anaesthetic was kept up to a definite level, and gradually dissociated as the level of chloroform pressure was allowed to fall, and as a result left the protoplasm free for a renewal of its metabolic processes—to choose a familiar analogy, that the protoplasm of a cell undergoing anaesthetization entered into a combination with the anaesthetic, similar to that between haemoglobin and oxygen, unstable in character, and only lasting so long as the pressure of the anaesthetic was kept up.

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[1] *Thompson Yates and Johnston Laboratories Report*, Vol. 5, Part I, p. 69.

It occurred to us, as protoplasm is built up chemically of proteid, that a certain amount of evidence as to the formation of such an unstable compound might be obtained, in the first instance, by experimenting with proteids.

We accordingly experimented with the proteids of the blood, and have obtained a number of results which together point to the formation of such compounds as are indicated above.

Our experiments may be described under the following headings :—

1. On the obvious physical and chemical changes produced in serum and in haemoglobin solution by the addition of chloroform.
2. On the relative solubility of chloroform in water, normal saline solution, serum, and haemoglobin solution.
3. On the relative vapour pressures of chloroform when dissolved in water, saline, serum, and haemoglobin solutions respectively, and on the variations in the coefficient of distribution in these solutions.
4. On the solubility of gases in serum and haemoglobin solution in presence of chloroform.
5. On the solubilities of other anaesthetics in water and in serum respectively.

I.—Effects of Chloroform on Serum and on Haemoglobin Solution

On adding chloroform[1] to either serum or haemoglobin solution and allowing the mixture to stand, changes occur which are obvious to the eye, and were to us previously unknown, but on consulting the literature we found that they had been observed by E. Salkowski[2] in using chloroform as a preservative for these fluids, and were also described by Formanek,[3] the bearing of such phenomena upon the question of anaesthesia was not, however, appreciated by these previous observers, who had approached the matter from a different standpoint, and as we have in some respects amplified their observations, and in others have obtained results not quite in accord with theirs, we feel justified in here recording our experiments.

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[1] The chloroform used for all the experiments described in this communication was presented to us by Messrs. Duncan and Flockhart, of Edinburgh.

[2] *Deutsche Med. Wochensch.*, 1888, No. 16; *Zeitsch. f. physiol. Chem.*, Bd. XXXI, 1900, S. 329. The fact that the red corpuscles combine with chloroform is also mentioned by Schmiedeberg, *Arch. f. Heilkunde*, 1867, S. 273.

[3] *Zeitsch. f. physiol. Chem.*, Bd. XXIX (1900), S. 416.

It was observed by E. Salkowski in 1888 that blood could not be preserved by adding chloroform, because it gradually became converted into a thick mass.

In 1891 it was observed by Horbaczewski[1] that haemoglobin was precipitated from a solution containing it, and kept at a temperature of 40-50° C., to which chloroform was added as a preservative.

The subject was investigated more minutely by Formanek[2] in 1900, and this observer found that a solution of haemoglobin kept at 50-55° C. for some time with chloroform was completely precipitated, the filtrate being entirely free from haemoglobin.

Formanek dried and analysed the precipitate, and from the absence of chlorine after fusion with sodium carbonate and potassium nitrate came to the conclusion that the precipitate is not a chloroform compound of haemoglobin. In our opinion this is not a valid proof, as the chloroform need not be so stably combined with the chloroform as to stand drying at 130° C., and subsequent fusion as employed by Formanek. The precipitate was dissolved by Formanek after thorough washing to remove the chloroform by the addition of a few drops of sodium carbonate solution, and the solution gave the bands of oxy-haemoglobin, and on treatment with ammonium sulphide reduced haemoglobin. Formanek also found that blood serum and egg-albumin were precipitated (when the reaction of the fluid was acid or neutral) on keeping at a temperature of 50-55° C. in presence of chloroform. From these experiments this observer came to the conclusion that the precipitate with which he was dealing was a mixture of haemoglobin and other proteids thrown out of solution by the chloroform. It is also stated in this paper that oxy-haemoglobin is only slowly and incompletely precipitated by the action of chloroform at room temperatures.

E. Salkowski, in his later paper,[3] states that blood kept at a temperature of 40° C. for 24-48 hours in presence of chloroform changes to a thick mass, but found that the precipitation of the haemoglobin was not complete under such circumstances.

Regarding the action of chloroform on serum, he states that serum can be preserved in contact with chloroform for years without precipitation at room temperatures, and finds this in agreement with Formanek's results, who found, in presence of an alkaline reaction, no precipitation of serum by chloroform even at a temperature of 50-55° C. Formanek does not state whether his alkaline reaction is the natural alkaline reaction of the serum. Salkowski also found a precipitating action of chloroform upon solutions of albumose and casein.[4]

Our experiments were conducted with haemoglobin and serum obtained from pig's blood. The serum used was obtained from clotted blood, and was thoroughly centrifugalized before use. The haemoglobin was in all cases obtained by centrifugalizing the blood corpuscles three times with normal saline, and then laking with distilled water and making up to the same volume as that of the blood taken.

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[1] Quoted by Formánek, *loc. cit.*

[2] *Zeitsch. f. physiol. Chem.*, Bd. XXIX (1900), S. 416

[3] *Zeitsch. f. physiol. Chem.*, Bd. XXXI (1900), S. 329.

[4] The chemical properties of the precipitate caused by the chloroform have been investigated by E. S. Edie, B.Sc., and the results obtained are recorded in the paper immediately following this, p. 195.

In the case of serum, we found that the fluid acquired, with less than one per cent. of chloroform (and greater quantities up to saturation), a peculiar opalescent and fluorescent appearance, but remained quite transparent to transmitted light. On the addition of over two per cent. of chloroform, there is a tendency to precipitation even in the cold, and at the end of twenty-four to forty-eight hours there is a slight precipitate present, but the effect is much hastened on placing the mixture in an incubator at 40° C., so that it becomes impossible to determine the maximum solubility of chloroform in serum at body temperature. Both in obtaining precipitation in the cold and more rapidly at 40° C. in presence of the natural alkaline reaction of the fluid, our results are at variance with those of FORMANEK and SALKOWSKI. The results were obtained several times in succession.

The marked opalescence in the serum was obtained in preparation of solutions of known concentration in chloroform for purposes of measurement of their vapour pressures, and led us to doubt at first whether we were not dealing with a fine emulsion of chloroform in the serum. Since this point was of vital importance to our experiments on vapour-pressure, we investigated it as completely as possible.

In the first place, examination with the microscope of the opalescent fluid showed no visible globules of chloroform, even with the highest powers.

To make certain of the matter, a current of air from an aspirator was bubbled first through chloroform contained in a WOULFF's bottle, afterwards through a similar bottle containing water, and then, *at the same temperature*, was sent through a third WOULFF's bottle containing serum. By this procedure the serum never came in contact with fluid chloroform, nor with air more highly charged with chloroform vapour than corresponded to the saturation of the air in contact with it or passed through it.

There could, hence, be no condensation of chloroform, and no means by which an emulsion of chloroform, finer even than could be seen with a microscope, could be formed.

Very soon, however, after the chloroform vapour began to pass through, a distinct difference in appearance was observable between the serum and a control placed alongside, and after a time the serum charged with chloroform in this manner was as opalescent as the specimens made in the usual way by shaking with weighed quantities of chloroform, and gave similar results with regard to vapour pressure.

These results show that the marked opalescence is not due to an emulsion of chloroform, and, further, that it is not due to precipitation of proteid in the ordinary sense of the word, for no precipitate can be seen with the microscope in the opalescent fluid.

Further, experiments on the vapour pressure show that the value of this is a long way from the maximum value, which it must obviously possess if an emulsion is present.

A similar opalescence is obtained on adding to serum other organic liquids which possess anaesthetic properties, thus we have obtained it on saturation of serum with ether[1], benzol, xylol, amyl acetate, amyl alcohol, and ethyl acetate, but have not followed the matter up as we have done with chloroform.[2]

In the case of haemoglobin solutions, we have not been able to observe an opalescence similar to that seen in the case of serum, up to the strength at which precipitation begins. In order to study where precipitation commences it is necessary to keep the haemoglobin and chloroform constantly stirred, or otherwise before the chloroform has dissolved the lower layer of haemoglobin solution in contact with the liquid chloroform becomes precipitated. When precautions are taken to prevent this occurring, precipitation is found to take place when about two per cent. of chloroform has been added, that is long before saturation is reached (*vide infra*). This precipitation prevents both the determination of the solubility of chloroform in haemoglobin solutions, and the observation of the vapour pressure with high concentrations. With concentrations of one per cent. or under no precipitation whatever was found to occur. Contrary to SALKOWSKI and FORMANEK, we found that no raising of the temperature was required to cause precipitation of the haemoglobin ; in fact, with strong solutions, the precipitation occurs within a few hours, even in the ice chamber. With time the precipitation is complete, and the precipitate is insoluble in water or saline, but in dilute sodium carbonate it is easily soluble, and we have found that the solution then shows the spectrum of alkaline haematin and not that of oxy-haemoglobin as was found by FORMANEK.

II.—ON THE RELATIVE SOLUBILITY OF CHLOROFORM IN WATER, SALINE, SERUM, AND HAEMOGLOBIN SOLUTION

In so far as we have been able to discover, no attention has been paid by previous experimenters to the maximum amount of chloroform capable of solution in the blood or serum as compared with that taken up by water or saline solution isotonic with blood.

When any reference is made to the matter, it has been usually assumed on general principles that the serum or plasma will behave like a saline solution of equal concentration and dissolve somewhat less chloroform than water[3]. In other words, that there is no specific action of the proteids or other substances in the plasma.

This supposition we have found experimentally to be entirely erroneous, for both serum and solutions of haemoglobin dissolve much more chloroform than water or normal saline solution. This fact is of importance in regard to the mode of action, as it definitely points to an interaction between the chloroform and the proteid present.

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[1] The opalescence with ether is not nearly so well marked, but increases on standing, and the solution in time becomes extremely viscid.

[2] See pp. 186-188

[3] See, for example, Overton, *Studien über die Narkose*, S. 98.

The presence of fats would, of course, increase the apparent solubility of chloroform in serum, and hence it is necessary in all cases to use perfectly clear serum, free from suspended fat; this precaution we have always been careful to observe; and, in addition, the serum has always been centrifugalized.

In this connexion, it may be added that the haemoglobin solutions which we have employed could not contain any appreciable amount of fatty matter, and hence the high solubilities which we have observed could only arise from chemical interaction between the haemoglobin and the chloroform.

Methods for Determining Maximum Solubility

Three methods have been used in the determination of the maximum solubility of chloroform in the solvents mentioned above, which have given concordant results, and shown that the solubility in proteid solution is much higher than in water or saline.

In the first method we have determined the amount of chloroform dissolved by obtaining the product of volume and vapour pressure at low pressure and with a small volume of fluid, so that practically all the chloroform was simply pumped off into the vacuum. In this method the volume of fluid experimented with is necessarily small, and this gives rise to experimental error of measurement, which is added to by the volume measured being large and pressure small, so that the results are only approximative, yet it is observable that they confirm those obtained by the more accurate methods described below.

The details of the method are described in the succeeding section on the relationship between vapour pressure and concentration of chloroform, and it need only be mentioned here that we have obtained solubilities of 0·95 per cent. in normal saline (0·75 per cent.), 3·33 per cent. in serum, and 4·42 per cent. in whipped blood by this method.

The second method employed consists in weighing out known amounts of chloroform into water and serum and haemoglobin solution respectively, and then determining by direct observation that concentration in each case at which the chloroform ceased to be dissolved.

This method of observation is made easy in the case of chloroform by the high specific gravity of that fluid, as a result of which, on inverting the flask in which the determination is being made, even minute globules of undissolved chloroform can be seen falling through the fluid.

The determinations of solubility by this method are made on the following plan: Pure chloroform is dropped from a fine capillary pipette into a tared graduated flask of 25, 50, or 100 c.c., and carefully weighed to definite amount, corresponding, when the flask has been filled by the solvent under experiment, to a definite percentage of chloroform. A series of such flasks is prepared, and immediately after each flask is filled with the desired solvent and either shaken thoroughly by hand until solution is complete, or placed on a rotary shaking machine. After the lapse of several days, during which time the flasks are never opened and are kept shaken up, it is noticed at what level of concentration the chloroform ceases to be completely dissolved, and so the solubility is determined.

The lower strengths of known value short of saturation and also the saturated solutions so prepared were kept and used also for the experiments on vapour pressure at varying concentration described in the next section of this paper.

The following results were obtained by the application of this method at room temperatures, approximately (13° C.), for the percentage by weight dissolved :—

Water, 0·8 per cent. dissolved, 0·9 per cent. dissolved, 1 per cent. not dissolved completely. Estimated solubility, 0·95 per cent.

Saline Solution (0·75 per cent. sodium chloride in water), 0·7 per cent. dissolved, 0·8 per cent. dissolved, 0·9 per cent. not dissolved. Estimated solubility about 0·83 per cent.

Serum, 3 per cent. dissolved, 3·5 per cent. dissolved, 4 per cent. all dissolved save a few small globules.

Haemoglobin Solution or Blood.—Over 6 per cent. by weight is taken up when chloroform is shaken with blood or haemoglobin solution of equal strength to the blood, prepared from blood by centrifugalizing several times with saline and subsequent laking with distilled water, and no globules of chloroform can be seen on careful examination with the microscope. But the solution rapidly changes in colour, and a precipitate is thrown out on standing, as above described, which is quite insoluble in water or saline, but easily soluble in dilute sodium carbonate solution, and then gives the spectrum of alkaline haematin.

The blood begins to give this precipitate when about 1·5 per cent. of chloroform has been added at room temperature, but with a lower concentration, and more rapidly when heated to body temperature in the incubator. Two per cent. of chloroform gives a precipitate in the cold, and on heating to 40° C. a red flocculent precipitate leaving a clear colourless fluid above.

The third method for determining the solubility of chloroform in the fluids experimented with consists in shaking up thoroughly for several hours with an excess of chloroform, and then pipetting off and determining the amount of chloroform in the solution.

The difficulty here is a rapid and accurate method of determining the amount of chloroform contained in a measured volume of the given saturated solution.

The procedure finally employed for this purpose was as follows :—

A measured volume (usually 10 c.c.) of the fluid saturated with chloroform is placed in a flask fitted airtight with a double bored cork, and a stream of hydrogen is aspirated through the solution, the oxygen present in the flask and connexions is absorbed by passing through alkaline pyrogallate, and the mixture of hydrogen and chloroform is then burnt by passing over heated palladium asbestos placed in a very short combustion tube. All the chlorine in the chloroform is thus burnt to hydrochloric acid, and the amount of this absorbed in standard alkali is then estimated, first by back titration against standard acid, and then further checked, either by volumetric titration with standard silver nitrate solution or by gravimetric determination as silver chloride.

The serum used in these determinations was examined for chloroform emulsion by the microscope, but no undissolved chloroform in suspension was observed. The precipitate in serum at atmospheric temperature obtained by this method of shaking up with excess of chloroform was very dense, so that the serum became quite opaque.

The results obtained by employing this method were as follows :—

Distilled water, dissolved 0·95 per cent., and serum, dissolved 5·08 per cent.

III.—ON THE VAPOUR PRESSURE OF CHLOROFORM DISSOLVED IN VARYING CONCENTRATION IN WATER, SALINE, SERUM, AND HAEMOGLOBIN SOLUTIONS RESPECTIVELY

A determination of the vapour pressure of an anaesthetic in solution at varying concentrations in serum, in haemoglobin, or in blood, is of high practical importance, since it is upon the relationship of this vapour pressure to the concentration of the solution that the amount of anaesthetic taken up by the blood circulating through the lungs depends.

It has hitherto been taken for granted that the DALTON-HENRY law can be applied, and that the amount of anaesthetic taken up is strictly proportional to, and varies directly with, the percentage of the vapour of the anaesthetic in the inspired air.

This has never, however, to our knowledge, been experimentally tested, and it seemed to us desirable to attempt such a determination. We have investigated from this point of view solutions of chloroform in serum, haemoglobin solution (of equal strength in haemoglobin to the blood from which the haemoglobin was prepared) and whipped blood, and have contrasted the pressures obtained with those of solutions in chloroform, in water, and normal saline at equal concentrations.

The vapour pressures have been measured corresponding to concentrations ranging from considerably below the anaesthetizing values for chloroform vapour pressure in air (viz., 8-10 mm.), observed by PAUL BERT, up to the saturation points in most cases.

APPARATUS

The instrument employed for this purpose was a form of 'differential densimeter,' which, after passing through many modifications, took the form represented in the accompanying sketch (Fig. 1), which is drawn approximately to a scale of one-quarter.

The two tubes shown are exactly similar, and are graduated in cubic centimetres and tenths in the upper portion, and in centimetres in the lower and wider portion.

The tubes are connected as shown by means of thick-walled rubber tubing and a glass Y-piece to a stout glass mercury receiver capable of holding more than enough mercury to fill both tubes and their connexions.

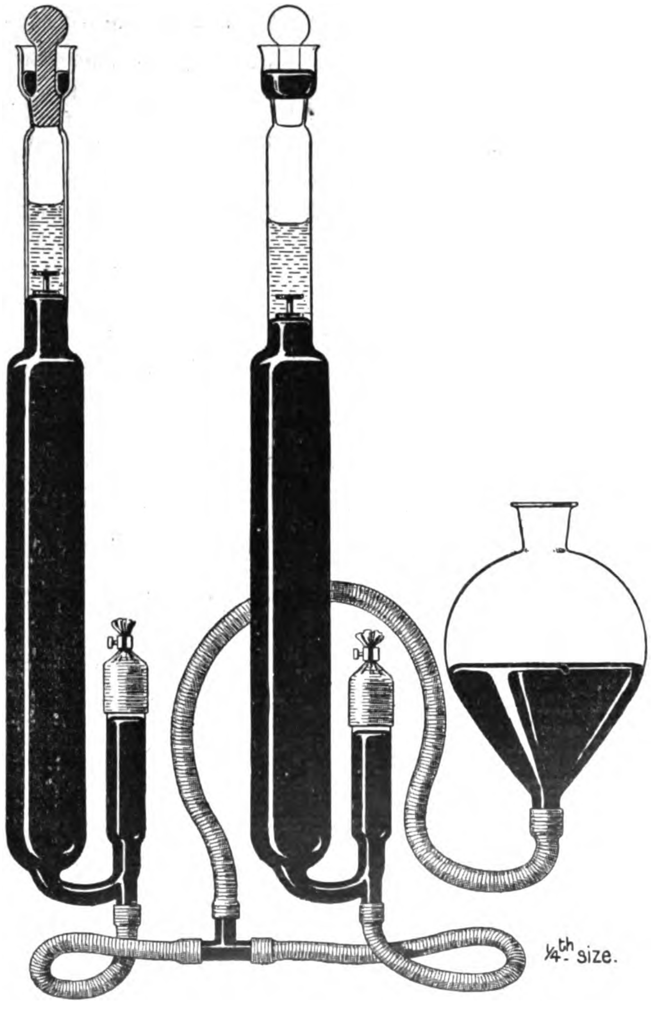

FIG. 1.—Diagram of Differential Densimeter.

The tubes are held in a vertical position by clamps attached to the strong vertical iron bar of a massive retort stand, and each tube is capable of being moved in its clamp vertically up and down for purposes of adjusting the mercury levels.

In order to keep a constant temperature (in the case of the experiments carried out at body temperature) the upper portion of each tube, from about the middle of the wide part to the level of the stopper at the top, was encased in a hot-water jacket of the form shown in detail in Fig. 2, and omitted for clearness from Fig. 1.

It was found convenient to use for the outer glass tubing of this jacket the largest size of a common variety of paraffin-lamp chimney, which measured 8 cm. in diameter above, bulged out as shown to 9 cm., and then narrowed below to 6 cm.

The wider top and bulge were found very useful in facilitating the introduction and vigorous movements of the bar electro-magnet used as a stirrer in connexion with the iron stud (seen in Figs. 1 and 2, at the top of the mercury), which was dropped into each tube, and, during an experiment, agitated up and down so as to thoroughly mix the fluid under experiment, and so bring it into equilibrium more rapidly with the vapour in the space above it.

The hot-water jacket was made watertight below by means of an india-rubber cork, as shown (Fig. 2); the rubber cork was also bored in each case for two narrow glass tubes, for the purpose of carrying water to and from the jacket. The two tubes carrying the water in stopped about 2 cm. above the upper surface of the rubber cork in each tube, so as to prevent blocking by mercury accidentally run over from the top when the inner tubes were being cleared out at the termination of a measurement. The outer ends of these two tubes were attached by means of narrow rubber tubings and a glass Y-tube to a bath of hot water, placed at a higher level, and a screw-down clip on each rubber tube regulated the flow until a thermometer placed in the corresponding hot-water jacket showed the desired temperature. A constant level of water was kept up automatically in the warm supply bath, and its temperature was regulated so as to lie 2-3° above that of the jackets. The two outflow tubes passed up, as shown, inside the jacket to the level of the ground in glass stopper, and their outside ends were connected by means of rubber tubing to the waste pipe.

In Fig. 1 the upper portion of the left-hand tube is shown in section, and that of the right-hand one in outline. The ground in stoppers shown were found, when sealed with mercury, to be much more effectual against minute leakages, which entirely vitiate the results, than any form of tap, and they are also much more convenient for introducing the solutions to be experimented upon, and for cleaning out the apparatus. Further, since they do not require to be operated between the commencement and termination of each determination of vapour pressure, they are better adapted to their particular purpose than a tap. In the course of our experiments, we also found it necessary to be able to dilute a solution with more of its solvent without allowing it to come in contact with any appreciable volume of air, and for this purpose found the stopper arrangement most convenient.

The side tube shown at the lower end of each main tube was designed to trap air which was found to slowly leak in through the rubber pressure tubing, when a vacuum was established by lowering the mercury receiver, and for a long time was a source of annoyance. We subsequently learned that the device had been first introduced by Lord Rayleigh. At the end of an experiment, any air which has collected is discharged by raising the mercury-holder, opening the screw-clip shown, and allowing enough mercury also to pass through to form a seal in the rubber tubing above the clip.

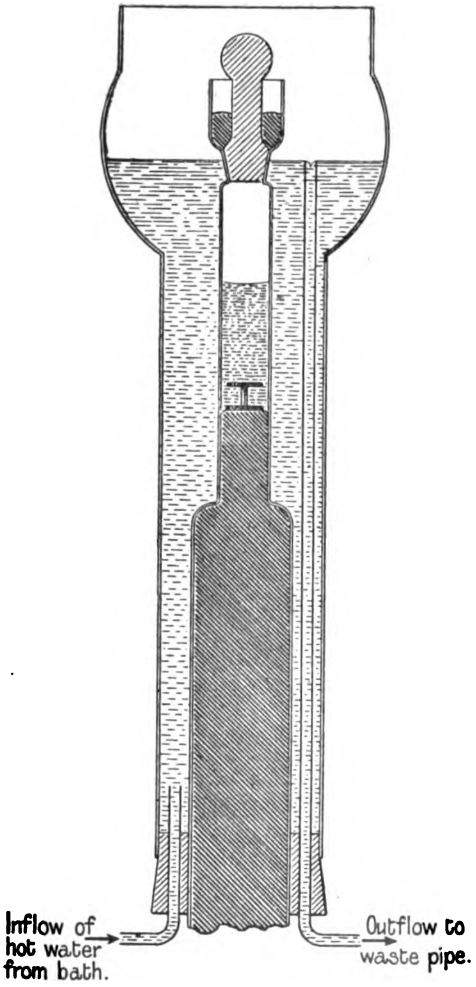

Fig. 2.—Section taken through the upper portion of one tube of the Differential Densimeter and the hot-water jacket, showing the inflow and outflow tubes. Scale, one-third.

The mercury-holder was suspended by means of a ring and loop of wire attached around the bulb from a vertical rod, swivel, and hook, possessing a slow screw movement in a block attached to a horizontal rod fixed in a clamp, which could be moved up and down on a heavy retort stand. For large movements, the clamp was slid up or down the retort standard, and for fine movements the screw was raised or lowered in its block.

In using the apparatus, the two vertical tubes are first placed at the same level, the mercury-holder is filled with mercury, and, with the two glass stoppers out, the whole apparatus is filled with mercury, the two stoppers are next inserted, enough mercury being left above them to form a seal, and the mercury-holder is then lowered until the two vertical tubes become evacuated. The receiver is then raised again to the level of the stoppers, and any bubble of air found is discharged.

The apparatus is now ready for an experiment, and, with stoppers out, the levels of mercury are adjusted until there is an equal volume left above the mercury on each side. A given volume of the solvent (say 5 c.c.) is now introduced on the one side (say left), and an equal volume of the solution of chloroform in the same solvent on the other side. In each case, immediately after the fluid has been introduced, the stopper is inserted, care being taken to prevent any air being included, either as a bubble at the mercury surface, or between the surface of the introduced fluid and the stopper. To achieve the latter end, we have almost always introduced above the mercury 2 or 3 c.c. more than the required quantity, so that it stood in the neck and slightly above, and then, by easing the stopper and gently adjusting the level of the mercury-holder, have brought the level of the mercury in the tube to the desired volume mark. After the solvent on the one side and the solution of chloroform of the desired strength on the other side have been successfully introduced in equal volume, and without any bubble of air, the mercury-holder is lowered until a space containing vapour has appeared on each side. The level of the mercury will be found to be lower on the chloroform side, and it is obvious, the instrument being independent of variations in atmospheric pressure, and the only different factor being the added chloroform on the one side,[1] that the difference in pressure will give the vapour pressure directly for that strength of chloroform solution at that particular temperature.

There is hence no need to determine pressure due to dissolved gases on the two sides,[2] or pressure of aqueous vapour, since these balance, and the quickness with which readings can be directly obtained makes it possible to carry out a long series of determinations at varying strengths, without the proteid solutions having time to undergo bacterial change.

Certain precautions have to be taken, however, and corrections made which may here be mentioned :—

1. Before taking a reading it is essential to move the tubes vertically and adjust the levels until the volumes of the vapour spaces above the upper aqueous solution meniscus in each case are exactly equal, otherwise inequality in pressure of gases pumped off on the two sides gives rise to an error, which is greater the smaller the vapour space.

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[1] There will be a small difference in the pressure of water vapour on the two sides, due to there being a stronger solution on the chloroform side, but this is in all cases too minute compared to the pressure of the chloroform vapour to make any appreciable error.

[2] Slight differences in dissolved gases gave a disturbance with very dilute chloroform solutions, and this was later obviated by pumping the gases off (*vide infra*).

2. Before taking a reading, there must be certainty that each fluid is in equilibrium with its vapour space. This is shown by absence of variation when the apparatus is left at rest.

For rapid and accurate working the mechanical stirring by means of the studs and magnet is indispensable, for even after the lapse of an hour when at rest the solution has not completely discharged its proper amount of chloroform into the vapour space. When once the control, containing, of course, no chloroform, has been thoroughly stirred it remains constant, and need not be changed at the end of each determination, but can be used throughout an entire experiment.

By vigorous stirring, equilibrium can be attained in five to ten minutes, and the level does not afterwards change, no matter how long stirring, and observation be kept up. This important experimental observation we have taken occasion to verify several times during our experiments.

3. For very accurate working, especially with the dilute solutions and low pressures, it is necessary in the case of serum and haemoglobin to pump off the dissolved gases by means of a Töpler pump, otherwise these come off unequally from solvent and solution and disturb the results at the low pressures. The chloroform solutions are then made up from the pumped-out solvent, which also must be used for control and for making the dilutions.

4. The temperature must be the same in the jackets surrounding each tube at the time when each reading is taken, and in a series of determinations at varying strength and a constant temperature, that temperature must be closely maintained throughout. The temperature error is a maximum when the solutions are near saturation, for then the variation in vapour pressure per degree is very large; fortunately here the differences in level under observation are also very large, which diminishes the percentage error arising from small deviations in temperature.

At concentrations away from saturation, the variations arising from small differences in temperature approximately obey the gas law, and under the conditions of our experiments become quite negligible.

5. A correction must be made in all cases upon the concentration of the solution introduced into the tube for the amount of chloroform pumped off from the solution into the vapour space. This correction is, of course, larger in the case of the more concentrated solutions with high vapour pressures.

This has been done in the experiments of which records are given below, and accounts for the concentrations not being exact percentages or small fractions of exact percentages.

The amount of chloroform in the vapour space is readily calculated from the product of the observed vapour pressure and the volume of the vapour space, and this amount deducted from the quantity contained in the chloroform solution when it was introduced, gives the necessary datum for calculating the concentration in chloroform of the solution corresponding to the observed vapour pressure in the vapour space.

The ratio of the vapour concentration in the fluid to the concentration in the vapour space gives the coefficient of distribution (*coefficient de partage*, *Theilungscoefficient*) ; this should remain constant if the absorption of the chloroform vapour by the liquid were normal and strictly proportional to the vapour pressure, and if it varies it points to a physical or chemical aggregation or compound between the chloroform and the fluid or its constituents (*vide infra*).

Method of Reading.—The readings were taken with a cathetometer,[1] placed about four feet from the tubes, both for greater accuracy in reading than direct measurement would give and to avoid changes in temperature.

Two Methods of Experimentation.—We have employed two different methods of experimentation in investigating the variation in vapour pressure with varying concentration. It is obvious that the concentration of a measured volume of a strong solution introduced into the densimeter may be diminished by pumping off more and more chloroform, by increasing the volume of the vapour space above the solution.

A series of readings of differences in pressure may thus be obtained in which the vapour space on the two sides is kept equal and of known and increasing value throughout the series. This method we have called the method of *variable vapour space.*

On the other hand, a series of solutions of known and steadily diminishing or increasing concentration may be introduced into the densimeter and measured one after another as to their vapour pressure, in each case with a known fixed volume of vapour space. As stated above, the concentration at which each vapour pressure in the series is measured is then accurately known. This method we have called the method of *constant vapour space.*

Method of Variable Vapour Space.—In this method, unless the volume of the tubes of the densimeter is very large, the volume of solution and solvent respectively introduced must be very small. We have usually taken $\frac{1}{2}$ c.c. on each side, either of a saturated solution or of a very strong solution of known strength, and, by altering the levels of the tubes and mercury receiver, have taken a long series of readings, in each case with equal volume of vapour space on each side, at every increasing volume of vapour space, until the difference in pressure became of small value, and the product of volume and vapour pressure became approximately constant, showing that practically all the chloroform had been pumped off from the solution. This constant product then gave the necessary datum for calculating the concentration of the original solution introduced into the densimeter, the product of the vapour pressure and volume at each stage gave the datum for calculating the quantity of chloroform pumped off from the solution, and, therefore, for deducing the corresponding concentration of solution. Or, also, by plotting vapour pressures as abscissae, and the product of vapour pressure and volume of vapour space as ordinates, the ratio of vapour pressure and amount of chloroform absorbed at each stage could be shown.

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[1] The instrument used was made by Pye and Co., of Cambridge, and, by means of a vernier and divided screw-head, read to $\frac{1}{100}$ mm. We have frequently observed that we were able to take readings within five divisions, that is $\frac{1}{20}$ mm., which is far within the accuracy of other portions of our determinations.

The method of *variable vapour space* has, however, two working disadvantages which caused us in the end to abandon it and replace it by the method of *constant vapour space*. The first objection is that the amount of solution taken is small, hence it is difficult to measure it with accuracy, and to make it equal on the two sides.

The second objection is that at the low concentrations the increase of volume for a small fall in pressure is very large, and hence the determinations become inaccurate, a small error in pressure-reading making a large deviation. The values for high pressures are also inaccurate., but for a different reason ; these readings are taken with small volumes of vapour space, and unless the vapour spaces are accurately equal on the two sides, there is a large disturbance due to inequality in pressure of the previously dissolved gases pumped off on the two sides.

The results, however, in the intermediate pressures are accurate and are given below, as they confirm those given by the other method.

Method of Constant Vapour Space.—In using this method we have always introduced a volume of 5 c.c. of the solvent on one side, and 5 c.c. of a solution of known strength on the other, and have invariably adjusted the levels so that at the temperatures of observation there was a vapour space of exactly 5 c.c. on each side.

In some cases we have started with a saturated solution of chloroform, and have then made dilutions of different percentages of that solution in the manner described below. In the later experiments we found it, however, more expedient, on account of knowing the exact concentration directly, to prepare a solution of known strength, say one per cent., and for the more dilute solutions to use various percentage dilutions of this stock solution. The more concentrated solutions were obtained by making up solutions varying by one per cent. in strength, and one-half per cent. differences were got by mixing these with each other in equal proportions. For percentages less than 0·1 per cent., a 0·1 per cent. solution was first prepared by making a ten-fold dilution of the one per cent. solution, and this 0·1 per cent. solution was then diluted similarly to the one per cent. solution.

PRECAUTIONS IN PREPARING, PRESERVING, IN UNALTERED STRENGTH, AND DILUTING BY KNOWN AMOUNTS, OF THE SOLUTIONS USED

In working with the solutions it is indispensable that due precautions be taken against loss by escape of chloroform into the air during the various manipulations.

If a portion, for example, of a stock solution of known strength be pipetted off for use in the densimeter, and then the stock bottle be merely stoppered, the air space over the portion of solution left in the bottle rapidly becomes charged with chloroform vapour at the expense of the stock solution, and a second sample taken later from the same bottle will be found to be weak and give a wrong result. The way to guard against this is to fill the bottle with mercury up to the neck immediately after drawing off, and at once stopper up. Similar devices were employed in all dilutions to complete the process out of contact with air.

The stock solutions were made by direct weighing, by dropping from a fine pipette into graduated glass-stoppered flasks of 25, 50, or 100 c.c. capacity, according to the amount required, immediately filling up to the mark with the particular solvent, and setting at once upon a slowly rotating disc, driven at such a rate that the chloroform globules have just time to fall through the solution each time the flask is inverted. In this way a rapid solution is effected, so saving much time. Further, in certain cases such shaking is indispensable, for a solution of haemoglobin left in contact with even a small amount of chloroform without continuous shaking soon forms a precipitated layer at the bottom.

The stock solutions were kept carefully stoppered, unless at the moment of introducing the pipette to remove a portion for experiment, and then the space within the bottle was always filled to the stopper with mercury in the manner above described.

We found much difficulty owing to leakage of chloroform during dilution in the case of the strengths intermediate between the stock strengths, until it occurred to us to make these dilutions in the densimeter itself. For this purpose we always placed the mercury level at 10 c.c. below the stopper on the side of the densimeter in which the mixing was to be carried out, then the proper amounts of the two solutions necessary to give the desired strength was drawn up in two graduated pipettes and run into the densimeter tube, the stopper was then inserted, including only a minute bubble of air, and now by thorough agitation of the iron stud through the fluid by means of the electro-magnet a thorough mixing of the two fluids was attained, then by raising the mercury-holder and slightly easing the stopper 5 c.c. were allowed to escape, the mercury level being placed accurately at 5 c.c. A seal of mercury was finally dropped in above the stopper, and so the dilution was effected.

For example, to obtain a dilution of 1·5 per cent., 5 c.c. of one per cent. solution and 5 c.c. of two per cent. solution were drawn off into pipettes, placed in the densimeter and mixed as above described; to obtain a 0·4 per cent. solution, 6 c.c. of the solvent were taken and 4 c.c. of a one per cent. solution, and similarly treated; to obtain a 0·03 per cent. solution, 7 c.c. of solvent and 3 c.c. of 0·1 per cent. solution were taken, and so on.

Certain of our experiments were carried out at room temperature and others approximately at body temperature (40° C.); the following protocols and accompanying curves show some of the typical results obtained, which have been confirmed in most cases by duplicates :—

VARIABLE VAPOUR SPACE

Experiment I.—Distilled water containing approximately 0·78 per cent. of chloroform. Half-a-cubic centimetre was introduced into each tube of the densimeter of the chloroform water on the one side and of the same distilled water without chloroform on the other. The temperature at which the experiment was carried out was 17° C., and the volume at which readings of pressure were taken varied from 2 to 50 c.c. The percentage of chloroform in the water was not known directly, but was calculated by extrapolation of the curve showing V.P., see Curve I, Fig. 3.

The following table gives the results of the experiment, which are also shown graphically in Curve I, Fig. 3, in which the abscissae show pressures of chloroform vapour and the ordinates the product of the volume and pressure of vapour. In Curve I, Fig. 4, the same experiment is shown with pressures of chloroform vapour as abscissae, and percentage of chloroform dissolved at each pressure as ordinates :—

EXPERIMENT I.—DISTILLED WATER

TEMPERATURE 17° C.

Volume of vapour space	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform in vapour space	Weight in grammes of chloroform in vapour space	Weight in grammes of chloroform in solvent	Percentage by weight of chloroform in solvent	Coefficient of distribution between vapour space and solvent
2	74·61	0·04957	0·00099	0·00293	0·586	1 : 11·8
3	60·62	0·04027	0·00121	0·00271	0·542	1 : 13·4
5	51·29	0·03408	0·00170	0·00222	0·444	1 : 13·0
6	47·69	0·03168	0·00190	0·00202	0·404	1 : 12·7
7	43·08	0·02862	0·00197	0·00195	0·390	1 : 13·6
8	38·91	0·02585	0·00207	0·00185	0·370	1 : 14·3
9	35·98	0·02390	0·00215	0·00177	0·354	1 : 14·8
10	34·36	0·02283	0·00228	0·00164	0·328	1 : 14·4
12	28·83	0·01915	0·00230	0·00162	0·324	1 : 16·9
15	26·16	0·01738	0·00263	0·00129	0·258	1 : 14·8
20	22·62	0·01503	0·00301	0·00091	0·182	1 : 12·1
25	17·65	0·01173	0·00293	0·00099	0·198	1 : 16·9
30	15·84	0·01052	0·00316	0·00076	0·152	1 : 14·5
35	14·37	0·00954	0·00334	0·00058	0·116	1 : 12·2
40	12·43	0·00835	0·00334	0·00058	0·116	1 : 13·9
45	11·87	0·00787	0·00354	0·00038	0·076	1 : 9·7
50	10·54	0·00700	0·00350	0·00042	0·084	1 : 12·0

Experiment 2.—Serum containing approximately 1·65 per cent. of chloroform, the amount being determined by extrapolation of Curve 2, Fig. 3. Experiment conducted in all respects similarly to Experiment 1. Graphic records of results shown by Curve 2, in Figs. 3 and 4.

EXPERIMENT II.—SERUM

TEMPERATURE 18° C.

Volume of vapour space	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform in vapour space	Weight in grammes of chloroform in vapour space	Weight in grammes of chloroform in solvent	Percentage by weight of chloroform in solvent	Coefficient of distribution between vapour space and solvent
1	115·63	0·07655	0·00077	0·00746	1·492	1 : 19·5
1·5	111·85	0·07405	0·00111	0·00712	1·424	1 : 19·2
2	104·27	0·06904	0·00138	0·00685	1·370	1 : 19·9
3	98·15	0·06498	0·00195	0·00628	1·256	1 : 19·3
4	89·89	0·05763	0·00237	0·00586	1·172	1 : 20·4
5	87·05	0·05277	0·00264	0·00559	1·118	1 : 21·2
6	79·70	0·04975	0·00299	0·00524	1·048	1 : 21·7
7	75·14	0·04730	0·00331	0·00492	0·984	1 : 20·6
8	71·46	0·04487	0·00359	0·00464	0·928	1 : 20·7
9	67·16	0·04447	0·00402	0·00421	0·842	1 : 18·9
10	63·24	0·04187	0·00419	0·00404	0·808	1 : 19·3
12	58·05	0·03843	0·00461	0·00362	0·724	1 : 18·9
15	52·31	0·03463	0·00519	0·00304	0·608	1 : 17·6
20	43·64	0·02889	0·00577	0·00246	0·492	1 : 17·0
25	34·97	0·02315	0·00579	0·00244	0·488	1 : 21·1
30	32·38	0·02144	0·00643	0·00180	0·360	1 : 12·1
35	28·20	0·01867	0·00653	0·00170	0·340	1 : 18·2
40	25·44	0·01684	0·00674	0·00149	0·298	1 : 17·7
45	22·40	0·01483	0·00668	0·00155	0·310	1 : 20·9
50	21·03	0·01392	0·00696	0·00127	0·254	1 : 18·3
60	17·91	0·01186	0·00712	0·00111	0·222	1 : 19·0
70	16·03	0·01061	0·00743	0·00080	0·160	1 : 15·1
80	13·93	0·0092	0·00737	0·00086	0·172	1 : 18·7
90	12·12	0·0080	0·00722	0·00101	0·202	1 : 24·3
100	10·70	0·0071	0·00708	0·00115	0·230	1 : 32·4
125	8·77	0·0058	0·00726	0·00097	0·194	1 : 33·4
150	7·91	0·0052	0·00786	0·00037	0·074	1 : 14·3

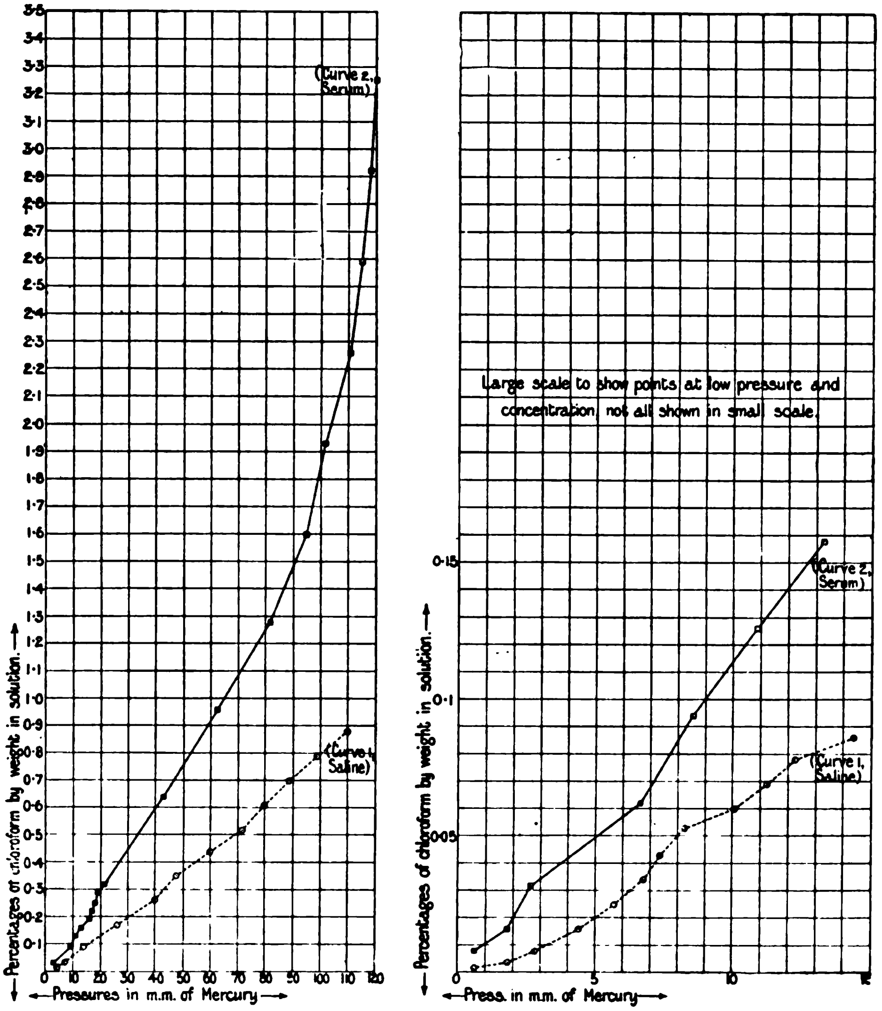
Experiment 4.—Serum containing approximately 3·33 per cent. of chloroform determined as in Experiment 3. Experiment performed similarly to Experiment 3, and temperature also 15° C. Results plotted in Curve 2, Fig. 5.

EXPERIMENT IV.—SERUM

TEMPERATURE 15° C.

Percentage by weight of chloroform originally introduced	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform pumped off into vapour space	Percentage by weight of chloroform remaining in solution	Coefficient of distribution between vapour space and solvent
0·0083	0·66	0·0004	0·0079	1 : 19·7
0·0167	1·82	0·0012	0·0155	1 : 12·8
0·0333	2·74	0·0018	0·0315	1 : 17·5
0·0666	6·66	0·0045	0·0621	1 : 13·8
0·0999	8·59	0·0057	0·0942	1 : 16·5
0·1332	10·85	0·0073	0·1259	1 : 17·3
0·1666	13·28	0·0089	0·1577	1 : 17·7
0·1999	15·54	0·0104	0·1895	1 : 18·2
0·2332	16·96	0·0113	0·2219	1 : 19·5
0·2665	18·37	0·0123	0·2543	1 : 20·7
0·2998	19·19	0·0128	0·2870	1 : 22·4
0·3331	22·49	0·0150	0·3181	1 : 21·2
0·6662	43·02	0·0281	0·6381	1 : 22·7
0·9993	63·15	0·0422	0·9571	1 : 22·7
1·3324	82·16	0·0550	1·2774	1 : 23·5
1·6655	95·30	0·0638	1·6017	1 : 25·1
1·9986	101·64	0·0680	1·9306	1 : 28·4
2·3317	110·53	0·0740	2·2577	1 : 30·3
2·6648	115·30	0·0771	2·5877	1 : 33·6
2·9979	117·53	0·0786	2·9123	1 : 37·1
3·3310	120·41	0·0810	3·2500	1 : 40·1

FIG. 5



Large scale to show points at low pressure and concentration, not all shown in small scale.

Experiment 6.—Serum containing amounts of chloroform shown in table by direct weighing. Results shown in Curve 2, Fig. 6.

EXPERIMENT VI.—SERUM

TEMPERATURE 40° C.

Percentage by weight of chloroform originally introduced	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform pumped off into vapour space	Percentage by weight of chloroform remaining in solution	Coefficient of distribution between vapour space and solvent
0·04	6·80	0·0042	0·0358	1 : 8·5
0·05	8·51	0·0052	0·0448	1 : 8·6
0·06	11·64	0·0072	0·0528	1 : 7·3
0·08	15·09	0·0093	0·0707	1 : 7·6
0·1	19·74	0·0122	0·0878	1 : 7·2
0·15	34·12	0·0210	0·1290	1 : 6·1
0·2	47·27	0·0291	0·1709	1 : 5·8
0·3	77·43	0·0477	0·2523	1 : 5·3
0·4	91·46	0·0563	0·3437	1 : 6·1
0·5	105·31	0·0663	0·4337	1 : 6·5
0·6	149·54	0·0921	0·5079	1 : 5·5
0·8	205·22	0·126	0·674	1 : 5·3
1·0	240·49	0·148	0·852	1 : 5·8
1·5	317·74	0·195	1·305	1 : 6·7
2·0	329·75	0·203	1·797	1 : 8·9
2·5	339·81	0·209	2·291	1 : 10·9
3·0	332·36	0·205	2·795	1 : 13·6
3·5	332·00	0·204	3·296	1 : 16·2
4·0	357·08	0·220	3·780	1 : 17·2

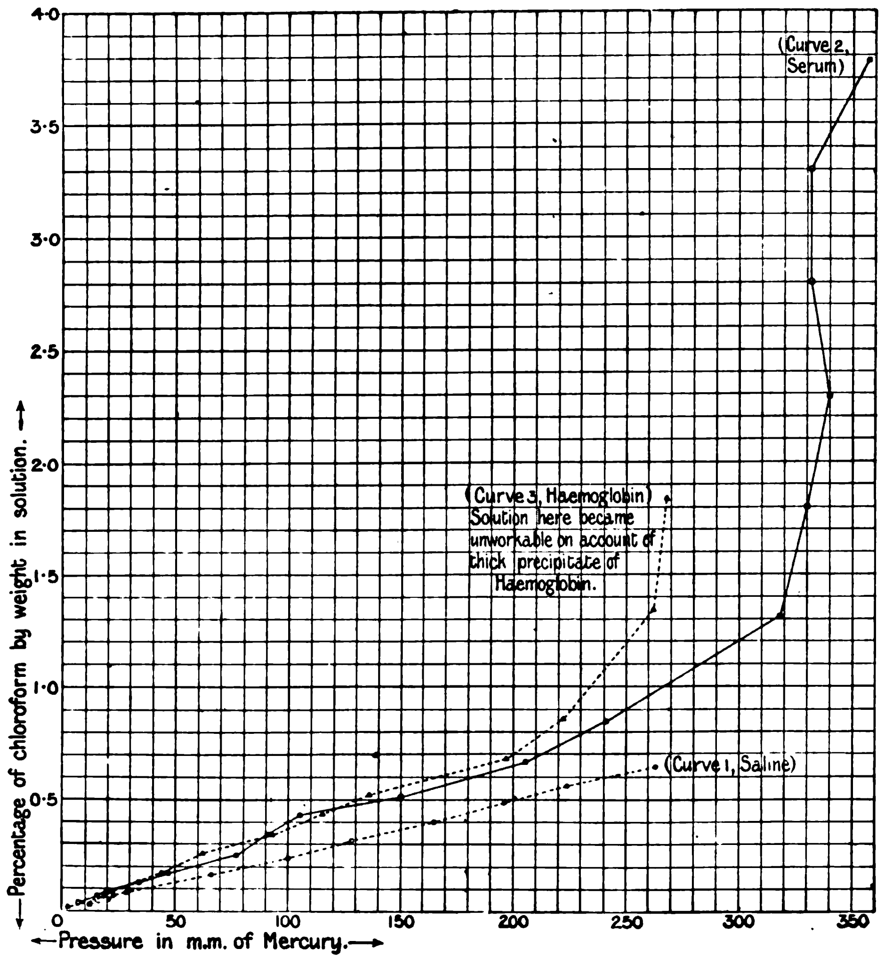
Experiment 7.—Solution of haemoglobin, of equal strength in haemoglobin to blood from which obtained, and containing the amounts of chloroform shown in the table. The results are shown graphically by Curve 3, Fig. 6.

EXPERIMENT VII.—HAEMOGLOBIN SOLUTION

TEMPERATURE 40° C.

Percentage by weight of chloroform originally introduced	Pressure of chloroform in vapour space in mm. of mercury	Percentage by weight of chloroform pumped off into vapour space	Percentage by weight of chloroform remaining in solution	Coefficient of distribution between vapour space and solvent
0·02	2·85	0·0018	0·0182	1 : 10·1
0·04	7·31	0·0045	0·0355	1 : 7·9
0·06	14·52	0·0089	0·0511	1 : 6·6
0·08	17·79	0·0110	0·0690	1 : 6·3
0·1	22·34	0·0138	0·0862	1 : 6·3
0·2	43·85	0·0270	0·1730	1 : 6·4
0·3	62·01	0·0382	0·2618	1 : 6·8
0·4	93·15	0·0573	0·3427	1 : 5·9
0·5	114·91	0·0707	0·4393	1 : 6·2
0·6	136·47	0·0840	0·5160	1 : 6·2
0·8	197·10	0·1213	0·6787	1 : 5·6
1·0	222·64	0·1371	0·8629	1 : 6·3
1·5	262·26	0·1614	1·3386	1 : 8·3
2·0	268·00	0·1650	1·8350	1 : 11·1

FIG. 6


(Curve 2, Serum)
(Curve 3, Haemoglobin) Solution here became unworkable on account of thick precipitate of Haemoglobin.
(Curve 1, Saline)
Percentage of chloroform by weight in solution.
4·0
3·5
3·0
2·5
2·0
1·5
1·0
0·5
0
50
100
150
200
250
300
350
Pressure in m.m. of Mercury.

It was found that the effects with all these substances, without exception, were comparable to those recorded above in detail for chloroform (Sections I and II).

In all cases the maximum solubility in serum is much higher than in water, and at a certain strength of added anaesthetic short of saturation alteration in character of the proteid commences, and finally leads to precipitation. The precipitation commences earlier, and is more complete in some cases than in others. In the case of ethyl ether the precipitation is slight, but the serum becomes gelatinous on standing.

The effects in each case may be briefly summarized as follows :—

Ethyl ether

Solubility in water at 15° C. = 8·0 per cent.
Solubility in serum at 15° C. = 11·0 per cent.

At ten per cent. and over there was a slight opalescence, and the fluid began to grow gelatinous. Eleven per cent. completely dissolved, twelve per cent. not dissolved and at higher percentages, two phases separated, the layer on top forming a clear, thin jelly.

Ethyl acetate

Solubility in water at 15° C. = 7·9 per cent.
Solubility in serum at 15° C. = 10 per cent.

At eight per cent., complete solution of the ethyl acetate and commencing precipitation of the proteid occurred. Solutions of nine and of ten per cent. strengths also dissolved completely, separation into two phases occurred at twelve per cent., the upper layer being viscid.

Amyl alcohol

Solubility in water at 15° C. = 2·4 per cent.
Solubility in serum at 15° C. = 8 per cent. or over.

At three per cent. complete solution of anaesthetic with commencing precipitation of proteid, this precipitation gradually increased through concentrations of 3·5 and four per cent. up to eight per cent., when a thick cream was formed. A separation into two phases could not be observed at any stage.

Amyl acetate

Solubility in water at 15° C. = 0·25 per cent.
Solubility in serum at 15° C. = 1·5 per cent.

At one per cent. complete solution of anaesthetic with precipitation of proteid, at 1·5 all dissolved except a few minute globules.

Benzol

Solubility in water at 15° C. = 0·15 per cent.
Solubility in serum at 15° C. = 0·6 per cent.

At 0·5 per cent. commencing precipitation and opalesence, 0·6 per cent. completely dissolved, 0·7 per cent. not all dissolved.

Xylol

Solubility in water at 15° C. = 0·016 per cent.
Solubility in serum at 15° C. = 0·2 per cent.

At 0·1 per cent. complete solution, cloudy from proteid precipitation, 0·2 per cent. dissolved, 0·3 per cent. not dissolved, 0·4 per cent. not dissolved. It is clear from the above results that a similar action and association between the anaesthetic and the proteid of the serum occurred in each case to that found in the case of chloroform. The amount of ethereal extractive in the different samples of serum used for the determinations varied between 0·24-0·36 per cent., thus demonstrating (see p. 191) that the increased solubilities found could not be due to solution of the anaesthetic in lipöid or ethereal extractives present in the serum.

Summary of Experimental Results, Discussion, and Conclusions

The experiments recorded above show :—

1. That chloroform and other substances possessing the properties of anaesthetics combine in either a physical or chemical manner with proteids in solution, and in so doing alter the properties of the proteid so that (i) the solution becomes opalescent or turbid, or it may be viscid, according to the anaesthetic employed, the change stopping short of actual precipitation of the proteid; or (ii) at higher concentrations there may be actual precipitation of the proteid which increases with rise of temperature, and may be either complete or incomplete.

Thus, chloroform, in the case of serum, causes a marked opalescence at concentrations far short of saturation, and at higher concentration a slow precipitation at room temperature (15° C.), and at body temperature, a rapid though incomplete precipitation. In the case of haemoglobin, one and a half to two per cent. of chloroform causes a change of colour and commencing precipitation at room temperature, which becomes almost complete in the thermostat at 40° C., while five per cent. and over causes complete precipitation even at 0° C.

Other anaesthetics have been tested and found to produce similar opalescence or turbidity, and a tendency to precipitation in serum.

2. That the maximum solubility of chloroform and a number of other anaesthetics in serum is much higher than in water or in saline of comparable strength to that of the fluids of the body. Thus, serum at a temperature of 13° C. was found to dissolve almost four per cent. of chloroform, while water dissolved less than one per cent. (0·95), and saline solution dissolved 0·83 per cent.

In the case of blood or haemoglobin solution, it is difficult on account of precipitation to estimate with any approach to accuracy the maximum amount of chloroform taken up, but six per cent. by weight may be added, and if rapidly shaken up is found to disappear so completely that no globules of chloroform can be found among the haemoglobin precipitate on careful microscopic examination. It has also been shown that a number of other anaesthetics have a much higher solubility in serum than in water.

3. That, for the same pressure of the anaesthetic at all concentrations, the amount of chloroform dissolved in serum or haemoglobin solution is considerably higher than in saline or water. Or, put in another form, at the same concentration of the anaesthetic in solution the pressure of the anaesthetic in a vacuum or air space is much less in the case of serum or haemoglobin solution than in the case of saline or water.

This has been shown for chloroform in concentrations varying from below the anaesthetization value to near the saturation point, and it has further been shown that while the curve of pressure and concentration in the case of water and saline is a straight line; in the case of serum and haemoglobin solution it is a curve showing association at the higher pressures.

The coefficient of distribution for chloroform at the anaesthetizing pressure (8-10 m.m.) and at 40° C. is, approximately, 4·6 for saline and 7·3 for serum, and at 15° C. these coefficients become 8·8 and 17·3 respectively.

4. The powers of haemoglobin and of serum for dissolving gases are not appreciably affected by the presence of the anaesthetic.

We believe that the experiments recorded above justify the conclusion that chloroform forms an unstable chemical compound or physical aggregation with the proteids experimented with, and that it is carried in the blood in such a state of combination. Since proteids build up the protoplasm of living cells, anaesthesia must be due, in our opinion, to the formation of such compounds which limit the chemical activities of the protoplasm. At low pressures of the anaesthetic, the compounds are unstable, and remain formed only so long as the pressure of the anaesthetic in the solution is maintained. Such compounds are formed not only by haemoglobin but by serum proteid, and hence the position taken by the anaesthetic in haemoglobin is not that of the respiratory oxygen. This is further shown by the fact that the oxygen-carrying power of haemoglobin is not interfered with in presence of chloroform.

Preliminary experiments with brain tissue and with cardiac muscle, both freed from blood, have demonstrated to us that these tissues possess a similar affinity for chloroform, as shown both by the higher solubility of chloroform in them, and at lower concentrations by the low pressures of the anaesthetic as compared with solutions of equal strength in water or saline.[1]

Since both haemoglobin and, in a lesser degree, even clear serum contain a small percentage of lecithin and other bodies soluble in ether, it has been suggested to us that the results we have obtained may be due to the solubility of the anaesthetic in these bodies and not to an action upon the proteids, and that the experiments should be repeated with proteids which are entirely free from lipöids.

In reply to this criticism, we would point out that in the first place it is a matter of great experimental difficulty, if not impossibility, to obtain proteids in solution unaltered and at the same time free from all trace of fat or lecithin ; and secondly, that a quantitative consideration of the conditions present in our experiments, as we shall proceed to demonstrate, makes it obvious that the small amount of lipöids present could not possibly account for the solubilities and absorption pressures which we have obtained.

Taking first the question of the preparation of lipöid free proteids, any precipitation with a neutral saline reagent will throw down mechanically the lipöid as well as the proteid, and in any process of extraction with a solvent, such as ether, the proteid becomes gradually altered in its properties, as above described. In the preparation of crystallized haemoglobin, to ensure that no lecithin is also mechanically present, repeated re-crystallization becomes necessary, and, as a result, the properties, including the solubility, of the haemoglobin in water become rapidly modified.

We have hence thought it better to obtain all our quantitative results with unattacked, unaltered proteid solutions, and the portion of our work dealing with the changes in physical properties of the solution, such as opalescence, turbidity, and, at higher concentratious, precipitation,[2] have left us in no doubt that the action is upon the dissolved proteid and not upon lipöids.

A quantitative consideration of the conditions in the solutions experimented upon also makes it very clear that it is the proteids and not the lipöids which are responsible for the low pressures and high solubilities.

The coefficient of distribution of chloroform between olive oil and water, according to Overton, is 30-33, and as those who support the lipöid theory use olive oil in their experiments as the representative of the lipöids, it would not be unfair to take as the basis of our calculations the above figure as the coefficient of distribution of the anaesthetic between the lipöids and water. Let us take, however, the higher figure of 40 as representing the coefficient of distribution for chloroform between the lipöids of the plasma and water, and calculate the percentage of lipöid which the plasma must contain in order that it may dissolve the amount of chloroform which we have observed on the assumption that none is dissolved or attacked by the proteids.

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[1] These experiments are in continuation, and the detailed results will appear in a subsequent paper.

[2] The large amount of chloroform in the precipitated proteid is clear evidence that the precipitation is due directly to a combination between the chloroform and the proteid. See Edie, p. 195.

According to the law of distribution, the amounts dissolved by the lipöids and by the water respectively will be proportional to the total amount of each present, and to the coefficient of distribution (40), and if there be x parts per cent. of lipöid and 100 − x of water, we have the following equation :—

$$\frac{x \times 40}{(100 - x) \times 1} = \frac{\text{Amount dissolved by lipöids}}{\text{Amount dissolved by water}}$$

$$\text{or } \frac{x \times 40 + (100 - x)}{100 - x} = \frac{\text{Total amount dissolved}}{\text{Amount dissolved by water}}$$

or in 100 c.c. of the serum—

$$\frac{39x + 100}{100 - x} = \frac{\text{Total amount dissolved in 100 c.c. of serum}}{\text{Amount dissolved by the water of 100 c.c. of serum}}$$

Now the solubility in serum is 4 per cent., and in water is 0·95 per cent., and substituting these values we obtain—

$$\frac{39x + 100}{100 - x} = \frac{4}{\frac{100 - x}{100} \times 0{\cdot}95}$$

$$\therefore 39x + 100 = \frac{400}{0{\cdot}95}$$

the value of x obtained from this equation is $x = 8{\cdot}23$. That is to say, in order to explain the solubilities which we have observed upon the lipöid theory, it would be necessary to suppose that the serum we used contained approximately 8·23 per cent. of lipöids.

We have found by experiment that the coefficient of distribution increases as the concentration rises, *for the higher percentages of chloroform*, and lies far above forty. On account of this change in coefficient of distribution, the amount of lipöid necessary to account for the solubilities observed is lower than that derived in the above calculation; but the amount of ethereal extractive in blood serum as a result of several determinations we have found to be only 0·24 to 0·36 per cent., and this amount is obviously entirely inadequate to account for the solubilities observed.

The same reasoning applies to all the other anaesthetics which we have tested, the increased solubilities cannot be referred to the action of the small amounts of lipöid in the serum, and can only arise from a union of some type between the anaesthetic and the proteid.

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